Gaps in Propolis Research: Challenges Posed to Commercialisation and the Need for an Holistic Approach

Shankar Katekhaye[a,b], Hugo Fearnley[b,c], James Fearnley[b,c], Anant Paradkar[a,*]

[a]*Centre for Pharmaceutical Engineering Science, University of Bradford, Bradford, BD7 1DP, UK*

[b]*Nature's Laboratory Ltd, Unit 3b, Enterprise Way, Whitby, North Yorkshire, YO22 4NH, UK*

[c]*Apiceutical Research Centre, Unit 3b, Enterprise Way, Whitby, North Yorkshire, YO22 4NH, UK*

[*]*Corresponding author- Tel.: 01274 233900*

E-mail address: a.paradkar1@bradford.ac.uk


Shankar Katekhaye

Shankar Katekhaye graduated in Pharmacy. He received his post graduate degree at NIPER, Mohali and doctoral degree in 2014 at Institute of Chemical Technology, Mumbai, India. Since May 2017, he has been working as a Knowledge Transfer Partnership (KTP) Associate (Quality Development Manager) between University of Bradford and Nature's Laboratory Ltd., UK. He has more than four years post Ph.D. research, industrial and teaching experience. His main research interests are natural products and the development of quality standards for Herbals and Propolis.


Hugo Fearnley

Hugo Fearnley is a Director of Nature's Laboratory Ltd, a leading natural products company. He is also a Director of the Apiceutical Research Centre. He originally trained in languages (Mandarin) and has experience in the charity and public sector. Hugo is particularly interested in the potential of beekeeping as a tool for social and economic development. He has focused on developing the Bee Pharma Africa project for Apiceutical Research Centre and recently completed a Winston Churchill Memorial Trust Travelling Fellowship as part of this development.

James Fearnley has been researching and manufacturing natural medicines, from both plants and the beehive for 30 years. He is CEO of Nature's Laboratory Ltd. In 2011 he set up Apiceutical Research Centre (www.beearc.com). He has worked particularly on exploring the chemical and biological activity of propolis. He is author of Propolis: Natural Healing from the Hive and Propolis and Oral Health. He has authored 12 peer reviewed research articles on propolis. He has coordinated the creation of the International Propolis Research Group (IPRG) who, along with ARC, is promoting international conferences on Propolis.


James Fearnley

Anant Paradkar gained his Ph.D. from Nagpur University, Nagpur, India. He is presently Director of the Centre for Pharmaceutical Engineering Science, University of Bradford, UK. He has been continually engaged in research activities for the last 30 years. His research interests include: process and product innovation, process understanding, hot melt extrusion, transdermal drug delivery, stabilised amorphous materials, polymeric micro-needles, and liquid crystalline systems.


Anant Paradkar

Gaps in Propolis Research: Challenges Posed to Commercialisation and the Need for an Holistic Approach

Both the season and region in which propolis is collected influence its chemical composition, resulting in variations in biological activity. Significant differences in composition and concentration of certain chemical compounds in propolis make standardisation and quality control challenging. In addition, the lack of uniformity in evaluation methodology and analytical techniques, make it extremely difficult to correlate data across the climatic zones. In this report, we focus on the gaps in propolis research and the challenges they pose for commercialisation, with suggestions as to how we might address them. We hope to stimulate further research which explores the holistic nature of propolis in order to derive a propolis bioactivity standard.

Key words: Propolis Activity Factor, Principle Component Analysis, Propolis Bioactivity Quotient, Honeybee, Composition-Activity Relationship, Contamination, Adulteration, Standardisation, Heavy metals.

INTRODUCTION

The word propolis was coined by Aristotle. It means pro (before) polis (city) i.e. before the city and is often referred to as defender of the city i.e. the bee colony. Propolis is also known as bee glue (Kuropatnicki, Szliszka, & Krol, 2013). It is a resinous material collected by bees from various plant exudates. Bees use propolis to narrow the hive entrance, seal cracks and embalm dead organisms inside the hive. The antibiotic property of propolis is a key factor in maintaining a healthy hive environment (Sanpa et al., 2015; Scazzocchio, D'Auria, Alessandrini, & Pantanella, 2006). Another important function of propolis in the beehive is to protect it against microbial invaders (José Maurício Sforcin & Bankova, 2011).

Whilst the precise composition of propolis varies greatly, it is generally composed of around 50% plant resin, balsams, plant latex, and vegetable glue, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances such as polyphenolic substances (e.g. organic phenols, flavonoids, and ketones) and organic debris (Burdock, 1998; Klaric et al., 2018; Monti, Berti, Carminati, & Cusini, 1983; Salatino, Fernandes-Silva,

Righi, & Salatino, 2011; J M Sforcin, 2007). Over 300 different compounds have been identified in propolis so far (Kasote, 2017), including broad groups of phenolics, flavonoids, and isoflavons, di- and triterpenes, cinnamates, coumarates, caffeic acids, prenylated derivatives, chalcones, lignans, and benzophenones. These chemicals in different concentration can be found in all three climatic zones. Propolis has been classified by researchers using a number of different approaches including type of propolis such as colour, chemical composition, geographical location, bee species etc (Anjum et al., 2018; Bankova, 2005b; Maraschin et al., 2016; Milena P Popova et al., 2007; Alexandra Christine Helena Frankland Sawaya et al., 2009; Yuliana, Wijaya, & Nasrullah, 2013). In this report, we have classified propolis into three distinct groups based on geographical location. Firstly, temperate zone propolis, originating principally from *Populus nigra* in Europe, North America, South Argentina, and non-tropical regions of Asia. This class of propolis is known to contain mainly flavonoid aglycones (flavones and flavanones), phenolic acids, and their esters (Bankova, 2005b; Bankova, Castro, & Marcucci, 2000; Bankova, Popova, Bogdanov, & Sabatini, 2002). The second type is tropical propolis from Central and South America, Central Africa, South and South-East Asia. This class of propolis is rich in phenolics, flavonoids, prenylated derivatives of *p*-coumaric acids, terpenes, lignans, and benzophenones (Bankova, Popova, & Trusheva, 2018; Marcucci, 1994; Salatino et al., 2011). The third type is subtropical propolis, from North Africa, Eastern Australia, Southern Africa, Northern Mexico, East coast of United States, and Mediterranean countries (Greece, Cyprus, Sicily, Croatia, Algeria and Malta etc). This type of propolis has a chemical composition similar to that of tropical propolis with additional constituents like aromatic acids, stilbenes, prenylated flavonoids and chalcones (Graikou, Popova, Gortzi, Bankova, & Chinou, 2016; Massaro, Simpson, Powell, & Brooks, 2015; Milena Petkova Popova, Graikou, Chinou, & Bankova,

2010; Sahinler & Kaftanoglu, 2005). In the above cases, the list of countries is not exhaustive (Figure 1).

Research over the last two to three decades has further exposed the wide potential of propolis, particularly its biological applications. Applications like anti-cariogenic (Eley, 1999; Koo, Rosalen, Cury, Park, & Bowen, 2002), anti-protozoan (Silva Cunha et al., 2004), anti-inflammatory (Borrelli et al., 2002), antioxidant (Ahn, Kumazawa, Hamasaka, Bang, & Nakayama, 2007; Isla, Nieva Moreno, Sampietro, & Vattuone, 2001), immune-stimulating (Oršolić, Knežević, Šver, Terzić, & Bašić, 2004; Sá-Nunes, Faccioli, & Sforcin, 2003), antiviral (Amoros et al., 1994), anti-diabetic (Al-Hariri, 2011), anti-tumor (Oršolić et al., 2004), hepato-protective (Banskota, Tezuka, & Kadota, 2001; Seo, Park, Song, Kim, & Yoon, 2003), anti-tubercular (Yildirim et al., 2004), antifungal (Ota, Unterkircher, Fantinato, & Shimizu, 2001) and antibacterial activity (Santos et al., 2002; J M Sforcin, Fernandes, Lopes, Bankova, & Funari, 2000; Silici & Kutluca, 2005), so it has been the subject of increasing scientific interest due to its diverse range of biological properties.

Whilst the literature illustrates the promising and wide ranging biological potential of propolis, we still do not have adequate and appropriate ways of understanding its quality parameters and of creating meaningful standards. At present, there is no credible standardisation model available. The complex chemical composition of propolis presents the greatest challenge to standardisation and this has prompted us to review the current status of propolis research and to review those challenges. In this article, we will explore the feasibility of creating a Propolis Bioactivity Quotient (PBQ).

CURRENT SITUATION

Trends in publication and research over the last five years

The principle theme of research over the last five years has been the biological evaluation of propolis rather than on standardisation and quality control. Standardisation has not attracted the attention of the scientific fraternity. However, some research groups notably Prof. Vassya Bankova, Bulgarian Academy of Sciences, Bulgaria and Dr. David Watson, University of Strathclyde, UK, have highlighted the importance of principle component analysis (PCA) as a potential tool for propolis standardisation. The search engine 'Scopus' was used to research the trend of publications on propolis. The Figure 2A illustrates the steady increase in the number of research publications on propolis. Research on Brazilian propolis has dominated publications over the last five years (Figure 2B) ("https://www.scopus.com/results/results.uri?," 2018).

The role of propolis in an era of drug resistance

The increasing reports of multidrug resistance (MDR) of pathogenic strains of bacteria to many available drugs and the slowing rate of new chemical entities being approved, continues to be alarming. Novel treatments for infectious diseases are urgently needed especially with the emergence of pathogens (e.g. MRSA, *Enterococcus* spp.) which have developed resistance to current treatment (Seidel, Peyfoon, Watson, & Fearnley, 2008). Propolis is well known for its antimicrobial activity against different bacteria, yeasts, viruses, and parasites by mechanisms such as direct action on microorganisms, and indirect action via stimulation of the immune system. Reports also suggest that propolis is effective against MDR bacterial strains (Carvalho, Silva-carvalho, Baltazar, & Almeida-aguiar, 2015; Freitas, Shinohara, Sforcin, & Guimarães, 2006; Gekker, Hu, Spivak, Lokensgard, & Peterson, 2005; Raghukumar, Vali, Watson, Fearnley, & Seidel, 2010; J M Sforcin et al., 2000; José Maurício Sforcin & Bankova, 2011). One study by Scazzocchio *et al*. demonstrated that propolis can

reverse resistance to some antibacterial drugs if used during the early stages of infection (Scazzocchio et al., 2006). Furthermore, *in vitro* experiments have revealed that propolis can work synergistically with the antibiotics (A. Fernandes et al., 2005; Scazzocchio et al., 2006; Stepanović, Antić, Dakić, & Svabić-Vlahović, 2003). In this context, we believe that the emergence of novel forms of antibiotic resistance could also be reduced by concurrent administration of propolis with antibiotics, paving the way for a new line of treatment strategy.

Legislative and regulatory environment

Twenty years ago, honey was by far the most important bee product within the European Union. Today propolis is also an important commercial product for beekeepers, along with other bee products e.g. bee pollen, royal jelly, beeswax, bee venom etc. Propolis is commonly sold as a food supplement and is considered a foodstuff of animal origin by the EU. Composition, labeling, and safety of bee based food products is regulated within the legislative framework of the EU (Vujić & Pollak, 2015). However, one EU report states that, the economic value of propolis is difficult to measure because it has no legal definition and is not a registered product (EC, 2013).

Polycyclic aromatic hydrocarbons (PAHs) are a group of more than 100 different chemicals, a number of which have proven carcinogenic properties. EU commission regulation No 835/2011 states the maximum level for PAHs in foodstuffs (EC, 2011). Reports on the high presence of PAH levels including benzo[a]pyrons above The European Food Safety Authority (EFSA) permitted level in dietary supplements, has caused alarm amongst propolis manufacturers (Moret, Purcaro, & Conte, 2010; Zelinkova & Wenzl, 2015). These reports may force EFSA to impose stricter regulation and in turn, restrict the sourcing of propolis from regions associated with high PAH levels. Currently we don't have enough information to clearly identify regions with high PAH levels in propolis. The increasing

research into propolis from different climatic zones, as seen in the case of Brazilian propolis, can only help the development of a more effective legislative framework in the near future.

CHALLENGES

Researchers and manufacturers face many challenges. These include standardisation, contamination, adulteration, regulation and the declining global bee population. The maximum permitted levels of contaminants, adulterants in propolis has not been established as in case of foodstuff. The commission of the European communities have set maximum levels for certain contaminants in foodstuffs, but no bee products could be found in the list (EC, 2011). Wide deliberation to formulate guidelines and regulations on establishing these limits is required. Such studies for bee products will help to define and establish the limits for contaminants in propolis.

Standardisation

It is possible that propolis is the most complex mixture of chemicals found in plant derived products. Various factors contribute to the chemical complexity of propolis such as phyto-geographical origin, climatic conditions, the time of collection, and the type of bees foraging etc (Bankova, 2005a; Burdock, 1998; Cheng & Wong, 1996; J. A. Fernandes, Leomil, Fernandes, & Sforcin, 2001; Murad, Calvi, Soares, Bankova, & Sforcin, 2002; Seidel et al., 2008; Silici & Kutluca, 2005). James Fearnley in 'Bee Propolis – Natural Healing from the Hive' (2001) states that the distinctive climatic conditions with their associated unique pathogens, force the local flora to develop those particular chemicals most useful for their own defence and survival (Fearnley, 2001). Advances in chromatographic analytical methods enables us to separate, extract and isolate the wide range of components from propolis (Kuropatnicki et al., 2013). Over 300 different compounds have been identified so far. The chemical complexities of propolis pose a real challenge to understanding content and

percentage uniformity, as well as predictable biological activity. Diverse chemical structures, molecular weight, and polarity of constituents make it difficult to apply a single analytical technique vis a vis standardisation even in today's era of very advanced techniques like LC-ELSD, LC-MS/MS, LC-NMR, GC-MS, and other hyphenated techniques.

Contamination

Pesticides

The primary source of pesticide residues in propolis continues to be agricultural practices as well as the application of pesticides in the hive. Insecticides, fungicides, herbicides, and acaricides have been detected in reports from different continents (González-Martín, Revilla, Vivar-Quintana, & Betances Salcedo, 2017; Mullin et al., 2010; Niell et al., 2015; Zhu, Schmehl, Mullin, & Frazier, 2014). Commonly used pesticides such as organophosphates, pyrethroids, carbamates, and organochlorides have been identified in propolis using various advanced analytical techniques. EFSA assessed and banned the use of clothianidin, thiamethoxam, imidacloprid, and fipronil after considering their effect on bees (EFSA, 2013). The European commission has decided to move towards implementing a complete ban on pesticides such as neonicotinoids, based on risk assessments by EFSA published in 2016.

Many steps are being taken to reduce the presence of pesticides in propolis and their detrimental effects on honey bee colonies. We will refer to some of them here with a focus on policy making, education, and awareness raising programs for beekeepers. In terms of policy making, some governments are identifying areas specifically for natural beekeeping, banning bee harming pesticides such as neonicotinoids, and labelling pesticide packaging with 'bee toxic' warnings. On the educational front, activities include, risk-reduction approaches for growers and pesticide applicators including recommendations for the use of pesticides only when needed, using less toxic formulations and less toxic compounds (particularly to avoid

broad spectrum insecticides, and application of pesticides during non-bloom phase of crops), avoiding contamination of water, and notifying beekeepers about pesticide application periods, making it possible to remove colonies in advance from such areas. Lastly, beekeepers can be made aware of the health hazards from pesticides to consumers as well as the impact on the beekeeping industry (Ellis et al., 2017).

Antibiotics

Abusive use of antibiotics and their wide spread presence in the environment where bees are foraging, means they find easy access into propolis. Accidental or sometimes intentional application of antibiotics like Tetracyclines and Chloramphenicol for disease control has contributed to alarmingly high concentrations in propolis (Bononi & Tateo, 2008; Levy, 1992; Zhou et al., 2009). Propolis is classified as a foodstuff of animal origin therefore, antibiotics residue in propolis is not tolerated in the EU (EC, 2000).

Heavy metals

Urbanisation, industrialisation and agricultural practices have contributed to high levels of pollutants in the environment. The toxic nature of heavy metals present in propolis is the most serious healthcare concerns for regulatory bodies. Honey bees accidentally collect heavy metals from the environment and carry them into the hive. Therefore, honey bees and hive products such as propolis can be used as indicators of environmental pollution. Because of the sticky nature of propolis, it is a better indicator of heavy metal contamination in the environment than honey (Conti & Botrè, 2001; Finger, Filho, Torres, & Quina´ıia, 2014; Matin, Kargar, & Buyukisik, 2016). The adverse effect of heavy metals and their prolonged half life in the body is a significant concern which may ultimately be detrimental to the future of propolis as a medicine.

A recent report on a technology using cellulose xanthogenate prepared from the common rush, and designed to remove excessive lead from propolis, is helping us to develop an economical and efficient technology to deal with this problem (L. H. Zhang, Li, Yuan, & Zhang, 2011). However, it is always better to prevent the entry of heavy metals into propolis, by avoiding beekeeping in industrial areas.

Adulteration

Increasing demand and limited supply of propolis has increased the practice of adulteration. A study on poplar propolis provides a good example of how propolis can be adulterated. *P. nigra* produces resin with a remarkable similarity in chemical composition, specifically flavonoids, to poplar propolis. This makes adulteration of propolis with poplar bud resin an easy task. This is well explained in research published by Zhang *et al*. where almost 27 out of 50 propolis products available in the market were found to be adulterated with poplar tree resin. Salicin, a marker compound, is found in poplar tree gum, but not in propolis. Salicin is hydrolysed by *β*–glycosidase present in the saliva of honey bees during propolis collection and processing (C. P. Zhang, Ping, Wang, Huang, & Hu, 2015). Recent reports studying adulteration in propolis have used the presence of salicin as a standard (C. P. Zhang et al., 2015). ~~Apart from using individual marker as detectors for adulteration, plant resins such as (poplar tree resin) can be detected easily in adulterated propolis (with high content of resin), as unadulterated propolis contains up to 50% resins.~~ In this respect, more studies are required which explore other useful marker compounds to detect adulteration in propolis.

Declining bee population

It is widely accepted that roughly one third of all food crops rely on natural pollinators particularly honey bees. Bees also produce hugely beneficial foods and medicines, such as honey, pollen, royal jelly, and propolis. Diminishing bee colonies can be linked to many

factors- biological, chemical and mechanical. The most important factor is biological, involving the invasion of honey bee colonies by the Varro mite, Irido virus, and Microsporidian. All are linked to bee colony decline (Bromenshenk et al., 2010). Colony collapse disorder (CCD) is the phenomenon that occurs when the majority of worker bees in a colony disappear and leave behind a queen, plenty of food and a few nurse bees to care for the remaining immature bees and the queen ("Pollinator Protection," 2017). This has been a particular cause of concern in United States with some beekeepers reporting unusually high losses of 30-90 percent of their hives (Environmental Protection Agency, 2017). In 17 European countries the average honey bee colony loss due to overwinter ranged between 2% and 32% (Jacques et al., 2017). Chemical and environmental factors are also a serious cause of decline. Environmental factors include, excessive use of pesticides (Neonicotinoids, a type of neurotoxin which basically attacks the bee's brain with a lethal dose of around 4 nanograms), contamination of the environment with heavy metals, antibiotics, and pollution in air and water. The third reason for decline involves mechanical factors including, large-scale monoculture, reducing diversity in cropping patterns, and adverse climatic changes producing both summer and over wintering losses. The reduction in foraging habitat of honey bees also increases their vulnerability. Clearly, honey bee health is multi-factorial and far more complex than originally thought.

GAPS IN PROPOLIS RESEARCH

Limited focus on propolis research

The most significant gap in propolis research lies in the limited number of reports and comparative studies which include both biological activity and chemical composition of propolis from different climatic zones (Bankova, 2005a). In addition, propolis research and publications has been focused on only a small number of countries. Most research has been

on Brazilian and European propolis. Recently there has been a surge in research into propolis from China, Turkey and India (Figure 2B). There have been few reports on African, New Zealand, and South Asian propolis. Propolis from tropical countries is still not well understood, although as discussed in the section on chemical complexity, we can expect promising biological activity and unique chemical composition attributable to the higher disease burden in these countries.

Another concern in connection with propolis research is that the rationale for conducting research studies often seems to be missing or misguided. This is presumably because research activities are uncoordinated and without clear and specific objectives. Many research groups are randomly screening for biological activity of propolis with limited or no correlation with chemical composition. Most of this research has to be excluded in any effort to construct a propolis activity factor (PAF) or measure of holistic standardisation.

The small number of clinical trial reports are also not encouraging. In spite of a reported plethora of biological activities, particularly anti-microbial effects, the trend of clinical trials suggests that, propolis has been mainly explored for oral problems such as mucositis (Piredda et al., 2017; Tomaževič & Jazbec, 2013), denture stomatitis (Pina et al., 2017), gingivitis (Bretz, Paulino, Nör, & Moreira, 2014), plaque inhibition (Kumar, Musalaiah, Pantareddy, & Sudhakar, 2015), dental hypersensitivity (Torwane et al., 2013), and cavity disinfection (Prabhakar, Karuna, & Deepak, 2015; Tulsani, Chikkanarasaiah, Siddaiah, & Krishnamurthy, 2014). A few clinical trials have been reported on other biological activities of propolis, such as wound healing (Henshaw et al., 2014); stomatitis (Samet et al., 2007), dengue hemorrhagic fever (Soroy, Bagus, & Yongkie, 2014), anti-*Helicobacter pylori* (Vaz Coelho et al., 2007), and reduced oxidative stress (Mujica et al., 2017) etc. Although propolis from almost all climatic zones is known for its anti-bacterial activity, it is surprising that so few clinical trials have been carried out. Propolis can reverse

antibacterial resistance, reduce the dose of antibiotics through synergistic mechanism and reduces the chances of the development of resistance to antibiotics (Wojtyczka et al., 2013). However, these facts need to be supported with clinical study data.

Inconsistencies in biological evaluation

Of the many biological activities of propolis, its anti-bacterial activity is most important in the context of the mounting problem of bacterial resistance to many drugs. Studies suggest that propolis is more active against Gram-positive bacteria (*Staphylococcus aureus*) than Gram-negative (*Escherichia coli and Pseudomonas aeruginosa*) and yeasts (Seidel et al., 2008; J M Sforcin et al., 2000; Silici & Kutluca, 2005). Although the results are promising, the methodologies used for evaluation vary widely in terms of their principles, research output, and measurement methodologies. It is very difficult to compare the results obtained from these methods. Four methods are most commonly used for accessing the antimicrobial effect of propolis. They are, bio-autography, agar diffusion, agar dilution and serial dilution assay (Seidel et al., 2008). In agar diffusion and bio-autography assays, results are reported as inhibition in millimeter (mm), whereas for broth dilution assay results are reported in minimum inhibition concentration (MIC) (Alexandra C H F Sawaya et al., 2004; Seidel et al., 2008). Apart from this principle difference, each method has its own limitations. The diffusion method is unreliable for comparing propolis samples, as results are directly influenced by the solubility of constituents in the agar media, owing to the highly complex mixture of compounds with variable polarity in propolis (Bosio, Avanzini, D'Avolio, Ozino, & Savoia, 2000; Alexandra C H F Sawaya et al., 2004; Seidel et al., 2008). Serial dilution in tubes (i.e. broth macro-dilution) has been considered to produce more consistent results and is more suitable for comparing different propolis extracts (Alexandra C H F Sawaya et al., 2004; Seidel et al., 2008).

Apart from variation in selection of method, the selection of microorganisms to test against also creates a challenge. If a universal factor for standardisation is to be derived, data used for biological evaluation needs to be gathered from studies where common microorganisms are used for example *S. aureus*.

Inconsistencies in analytical studies

It is technically difficult to integrate data where different analytical techniques which work on different principles are used to detect specific types of biochemical molecules (Ni et al., 2007). The following examples illustrate the limitations of some analytical techniques. The reference library pool for GC-MS focuses more on primary metabolites (organic and aliphatic acids, sugars, and amino acids) which have small molecular weight. In contrast, LC-MS cover large hydrophobic secondary metabolites such as alkaloids, terpenoids, and phenols (Liu et al., 2017). Therefore, when total content of these constituents are reported and quantified based on reference library pool; this can lead to discrepancies in the results. The stability of chemicals depends on the environment of the analysis, for example the process of alkylation of a sample as a part of sample preparation for GC analysis, transforms the flavanones into chalcones. This is supported by findings from the García-Viguera research group, where lower concentration of pinocembrin was observed in the same sample when using GC-MS rather than HPLC. The authors recommended HPLC over GC-MS for flavonoid analysis in propolis (García-Viguera, Ferreres, & Tomás-B arberán, 1993; Markham, Mitchell, Wilkins, Daldy, & Lu, 1996). However, HPLC, the commonly used technique for analysis, has its own limitations. Many constituents reported in propolis do not absorb ultra-violet (UV) light, therefore LC with UV detector cannot detect all the constituents. Diode-array and MS detectors are more advanced than simple UV detector, but neither can provide a complete solution. Very few studies have reported on the analysis of the same propolis sample using GC and LC, enabling us to see how different analytical

techniques affects the reported concentration of chemicals, such as flavonoids and phenolics. However, some research groups have performed analysis of propolis using a combination of these techniques (Park, Alencar, & Aguiar, 2002). More research needs to be performed using this approach.

IMPACT ON INDUSTRY

The difficulty in sourcing quality raw propolis

Research activity and production is often linked with the support and promotional policies of governments. It has been observed that the propolis from many locations has still not been studied for its biological potential and chemical composition. This makes it difficult to grade propolis from different climatic zones. Brazilian propolis is well studied and in the market is considered to be premium quality owing to its biological activities. Brazilian propolis is seen by some as a reference standard for comparative studies.

Another industry challenge is the availability of propolis. Apart from low productivity of propolis in the hive, many beekeepers remain unaware of its medicinal importance and economic potential. For most beekeepers, honey collection is their primary focus. Also, lack of education, training, and technology in propolis collection affects the industry. The gap in supply and demand alongside increased commercial value makes propolis susceptible to adulteration. For example, the practice of adulterating propolis with cheaper poplar tree resin creates a profitability challenge for companies selling genuine products. Poor quality products affect consumer confidence and eventually result in a lack of trust and reduced sales of products.

The challenge of quality assessment

Many leading scientists have argued that the biggest challenge for medicines originating from natural sources is quality control and standardisation. Bankova *et al*., state that the differences

in the composition of propolis from different continents, make it difficult to determine propolis quality and to standardise it (Bankova & Marcucci, 2000; Bankova et al., 2002). Lack of standardisation is a limiting factor in the acceptance of propolis as a viable alternative or complementary supplement to first line treatment of many diseases. This also hampers the growth of manufacturing.

To enter the regulated market and to comply with the strict rules for product registration and placement in such markets, demands optimised and validated products. The complex composition of propolis makes this a very challenging task and hence the biggest barrier to the expansion and growth of propolis products.

FUTURE AND REQUIRED INTIATIVES

Synchronise biological studies and chemical evaluation

Simultaneous evaluation of biological activities and chemical analysis of propolis is the most important research goal if we are to develop a meaningful and acceptable PAF. For biological evaluation, *S. aureus* is the most commonly used organism for screening anti-microbial studies. Uniformity in screening methodology and test bacteria would lead to more consistent results and better understanding of the efficacy of propolis from different climatic zones.

Using different analytical techniques on same samples simultaneously is paramount if we are to overcome their individual limitations and help to derive accepted parameters for standardisation. Earlier reports suggest that GC-MS should be the preferred analytical technique for analysis of non-flavonoid components (aliphatic and aromatic acids) and HPLC with diode-array detector should be preferred for the analysis of flavonoid components (García-Viguera et al., 1993; Markham et al., 1996). To minimise the limitation of HPLC, addition of ELSD with diode-array detector would be a better alternative.

In the end, to tackle these challenges, the collaborative research approach may be the best possible solution. Various research groups with different expertise can collaborate and explore propolis in a more logical way as has been seen in reports from some groups (Graikou et al., 2016; Kapare, Lohidasan, Sinnathambi, & Mahadik, 2017; Kasote et al., 2017; Ni et al., 2007; M. Popova et al., 2017; Seidel et al., 2008; Watson et al., 2006).

Integrated studies involving propolis from all climatic zones

Interestingly, in spite of the difference in composition, propolis from all regions, by and large, exhibit similar biological properties (Burdock, 1998; Marcucci, 1994). This directs us towards the possibility of determining the patterns of relationship between chemical composition and biological activities which would be applicable for propolis from most areas of the world. This idea is well supported with research published by Oruc et al., (2017) on partial standardisation of propolis where, compounds like galangin, naringenin, pinocembrin, pinobanksin, quercetin, apigenin, caffeic acid and caffeic acid phenylethyl ester (CAPE) were detected in all 45 samples from all seasons and altitudes (Oruç, Sorucu, Ünal, & Aydin, 2017). However, any such effort to derive a propolis activity factor using selective classes of markers would limit its application to propolis from specific regions. For example standards for assessing the quality of Brazilian propolis use isoliquiritigenin, medicarpin, Biochanin A and from Brazilian green propolis artepilin C, Drupanine, Bacharin etc. Propolins C and D are representative of prenylated flavonoids, major constituents of tropical propolis from Taiwan Okinawa and subtropical Australia (Chen, Ye, Ting, & Yu, 2018). It seems clear that, different classes of chemicals are related to specific biological activities. This fact suggests to us the need to identify separate propolis activity standards for different disease conditions i.e. an anti-oxidant standard, an anti-microbial standard, an anti-fungal standard and so on, each related to a specific set or family of chemicals. While setting propolis activity standard, the specific chemicals which are principally responsible biological effect of propolis has to be

considered while selecting the markers for deriving standards from different climatic zones. The characteristic constituents in temperate region propolis are flavonoids without B-ring substituents, such as chrysin, galangin, pinocembrin, pinobanksin and CAPE. In tropical region propolis, especially Brazilian green propolis, the dominating chemical components are prenylated phenylpropanoids (e.g., artepillin C) and diterpenes. For propolis produced in the Pacific region, geranyl flavanones are the characteristic compounds which are also found in propolis from the African region (Fernandes-Silva, Freitas, Salatino, & Salatino, 2013; Huang, Zhang, Wang, Li, & Hu, 2014). This would recognise and value the headline qualities of propolis from different climatic zones in a way that a single or universal standard could not do. More reports, integrating propolis from all the climatic zones would further help us to derive a robust PBQ.

Propolis bioactivity quotient classification

As a first step towards a series of propolis activity standards we propose a model for a propolis activity standard based on antioxidant activity. The standard would be based on a classification of propolis (I to VI) from high to low activity based on anti-oxidant activity (Figure 4). In time we can develop other PBQ classifications around other activities such as anti-microbial, anti-inflammatory, anti-cancer etc. We believe that such standards can be produced using multivariate analysis by SPSS for phenols flavonoids and other class of chemicals. This would help to design and classify propolis from different climatic zones. Classification would also help to cater for regional properties of propolis throughout climatic zones for specific biological activity.

Technologies to tackle the current challenges

In earlier sections, we have identified and highlighted some of the shortcomings in propolis research and the challenges they pose to industry. Some encouraging reports exist which can

help us tackle some of these challenges. Many technologies for the prevention and removal of contaminants e.g. pesticides, heavy metals, and antibiotics have been reported. Some of them are summarised below. Much of the research has focused on lead amongst other heavy metals in propolis. The purification of propolis using the alcohol-aqueous method can help to remove lead without losing its bioactive phenolics. In another technology, the wedge method seems to be the most appropriate technique compared to scraping and plastic nets to obtain the best quality propolis (Papotti, Bertelli, Bortolotti, & Plessi, 2012). However, mesh (plastic nets) is a preferred method of collection as it helps to reduce the level of lead in the propolis (Sales et al., 2006). A technology to remove excessive amounts of lead in propolis using cellulose xanthogenate, prepared from the common rush, has provided an economical and efficient adsorbent capable of removing heavy metals from propolis (L. H. Zhang et al., 2011). Paints have been identified as one of the sources of mercury, lead, cadmium and other heavy metals (Conti & Botrè, 2001). Educating beekeepers, particularly from developing countries to use metal free paints would remove this source of contamination. Simple, quick, and reproducible methods and technology, like dual-layer solid phase extraction, to clean-up contaminants like antibiotics, heavy metals, and pesticides offer technologies able to improve the quality of propolis (Oellig, 2016). Simple techniques like the filth-test can help to detect contaminants like arthropod fragments, mammal hairs (from rodents), carbon particles and inorganic fragments in propolis (Canale, Cosci, Canovai, Giannotti, & Benelli, 2014). It is recommended that beekeeping may be restricted to area at least 3 km radius to prevent lead and other heavy metal contamination in propolis (Bogdanov, 2006). The promotion and use of green technologies in collection, processing, removal of contaminants, and organic beekeeping can together offer a combined solution to the prevention and elimination of contamination in propolis.

Educating beekeepers would solve many of those problems created by lack of awareness. Greater awareness of the health hazards to consumers from pesticides, heavy metals, and antibiotics could better inform commercial beekeepers of best practice and thereby reduce contaminants, particularly antibiotics. The knowledge of advanced beekeeping, collections techniques, and hygiene management would also help.

Focus on the holistic approach

Kujumgiev et al. (1999) along with other research groups have demonstrated that investigations of the antibacterial action of individual substances, isolated from propolis showed that no single propolis component has greater activity than that of the total extract. Therefore, whole propolis should be administered as a natural mixture and we should not consider individual components as a source of a new powerful antimicrobial (Kujumgiev et al., 1999). Furthermore, Popova *et al*., suggested that the quantification of the active compounds into groups such as flavonol and flavones, having the same or close chemical structure, correlates better with the biological activity and is more informative than the quantification of individual components (M. Popova et al., 2004).

The best example which supports the aforesaid claim would be caffeic acid phenethyl ester (CAPE). This molecule has probably been explored more extensively than any other molecules in propolis. It has been identified with solubility and stability challenges. Intestinal permeability of CAPE when tested on Caco-2 cell (Gou et al., 2016), showed that the active drug transporter, P-Glycoprotein (Gou et al., 2016) was inhibited by CAPE. However, P-gp expression up-regulation after 48 h or 72 h exposure (Gou et al., 2016), suggest both increased efflux of CAPE and ultimately low concentration reaching the plasma. In addition to that, Caffeic acid ester has a water solubility (Demestre et al., 2009) and pH stability (Friedman & Jürgens, 2000) problem. In another published study, Narita *et al.* reported on the degradation of chlorogenic acid. Chlorogenic acid is a quinic acid ester of caffeic acid, while

CAPE is a phenethyl ester. The author stated that, the presence of ascorbic acid and epigallocatechin gallate with chlorogenic acid enhances its pH tolerance (Narita & Inouye, 2013). Therefore, the assumption can be made that, the stability and efficacy of CAPE and many other bioactive molecules in propolis extracts might be higher than their individual molecules owing to mechanisms such as synergism, stabiliser, bioavailability enhancer, and activity against multiple targets.

This prompts us to focus on developing an holistic approach to standardisation (Figure 3) which considers the important classes of chemical compounds. This can be called the bottom-up approach. However, working with standardised extracts will allow scientists to relate propolis with particular chemical composition to specific biological activities and therefore to formulate appropriate claims (Toreti, Sato, Pastore, & Park, 2013). Application of chemo-metric analysis (Tang et al., 2014), fingerprinting and principle component analysis (A C H F Sawaya et al., 2007) approaches need to be applied for better deciphering of the chemical composition of propolis. Finally, we suggest, a list of chemicals which can be termed biomarkers for standardisation of propolis based on frequency of occurrence and biological potential. Although any such effort to identify markers for standardization should consider chemicals unique to propolis from respective climatic zones. The few listed molecules include CAPE, chrysin, galangin, naringenin, pinocembrin, pinobanksin, quercetin, *p*-coumaric acid, ferulic acid, caffeic acid, cinnamic acid, isoliquiritigenin, medicarpin, biochanin A, artepilin C, drupanine, bacharin etc. which cover the vast majority of biologically active groups of compounds such as phenolics, flavonoid and isoflavons, phenylpropanoids, geranyl flavanones, cinnamates, coumarates, caffeates etc. In addition to above listed markers, one has to consider markers which are signature molecules to certain types of propolis from different climatic zones. Importantly, these classes of chemicals have

been reported from all the climatic zones across the globe in variable concentration. This approach can lead us towards designing PBQ.

SUMMARY

In summary, the last two to three decades have uncovered the potential of propolis via a diverse range of biological activities and unique chemical compositions. Various factors give rise to the chemical complexity of propolis, for example, phyto-geographical origin, time of collection, and type of bees foraging. Complex chemical composition of propolis is the most important reason for many of the analytical challenges. Apart from this, propolis has other challenges including lack of an holistic approach in research, contamination (pesticides, antibiotics, and heavy metals), adulteration, shortcomings of biological evaluation methodologies, and lack of clinical trials etc.

Amongst the plethora of biological activities, propolis is best known for its antibacterial potential. The research effort is lacking on two important fronts. Firstly, we need more credible data about reduced chances of antibiotic resistances by concurrent administration of propolis and antibiotics. Propolis can reverse antibiotic resistance and minimises the development of resistance for first line antibiotics. It also exhibits synergistic effect and can help to reduce the doses of antibiotics. Secondly, more clinical trials are needed to support the aforesaid claims. This would offer a new line of treatment strategy and a solution to the world wide crisis of antibiotic resistance.

The most important challenge faced by manufacturers is standardisation and the ability to obtain the right quality of propolis. The differences in composition of propolis from different continents make it difficult to both determine quality of propolis and to standardise it. Even with modern advanced analytical techniques, the diverse chemical structures, molecular weight, and polarity of chemicals in propolis make it difficult to apply a single analytical technique for quality assessment. Using two or more techniques is advocated.

Lack of comparative studies which combine biological and chemical evaluation poses a significant challenge to the standardisation of propolis. This could be due to random screening and limited collaborative research rather than focused screening and/or coherent research. Groups randomly screen the biological activities of propolis, with limited or no correlation with chemical composition. Simultaneous evaluation of biological activity and chemical analysis of propolis samples offers the best option for deriving a universal PAF.

In conclusion, our proposal is to develop a standardization protocol using an array of markers such as CAPE, chrysin, galangin, naringenin, pinocembrin, pinobanksin, quercetin, coumaric acid, ferulic acid, caffeic acid, cinnamic acid, isoliquiritigenin, medicarpin, biochanin A, artepilin C, drupanine, bacharin etc. which represent the majority of biological active groups of compounds such as phenolics, flavonoid and isoflavons, phenylpropanoids, geranyl flavanones, cinnamates, coumarates, caffeates etc. Bear in mind, above proposed list of chemicals is not exhaustive and important molecules has to be considered while performing such studies. This will enable us to identify the pattern and percentage of chemicals present in propolis from different climatic zones. Perhaps, it will allow scientists to connect a particular chemical profile to a specific from of biological activity. Furthermore, principle component analysis and the use of composition-activity relationship databases could guide us towards a propolis bioactivity quotient, which could be used for grading propolis by composition and for claiming specific biological activity. We hope that this article will attract attention for the concept presented and stimulate a new trail of thoughts on propolis research.

CONFLICT OF INTEREST

There are no conflicts to declare.

ACKNOWLEDGEMENT

We acknowledge the financial support from Innovate UK and Nature's Laboratory Ltd. for funding the project (KTP010490). We also acknowledge the financial support provided by Winston Churchill Memorial Trust for the project (2016-17).

REFERENCES

Ahn, M. R., Kumazawa, S., Hamasaka, T., Bang, K. S., & Nakayama, T. (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chemistry*, *101*, 1383–1392. https://doi.org/10.1021/jf048726s

Al-Hariri, M. T. (2011). Propolis and its direct and indirect hypoglycemic effect. *Journal of Family & Community Medicine*, *18*(3), 152–4. https://doi.org/10.4103/2230-8229.90015

Amoros, M., Lurton, E., Boustie, J., Girre, L., Sauvager, F., & Cormier, M. (1994). Comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-but-2-enyl caffeate. *Journal of Natural Products*, *57*(5), 644–647. https://doi.org/10.1021/np50107a013

Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Attaullah, M., … Dash, C. K. (2018). Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences*, *xx*, xx. https://doi.org/10.1016/j.sjbs.2018.08.013

Bankova, V. (2005a). Chemical diversity of propolis and the problem of standardization. *Journal of Ethnopharmacology*, *100*(1–2), 114–117. https://doi.org/10.1016/j.jep.2005.05.004

Bankova, V. (2005b). Recent trends and important developments in propolis research. *Evidence-Based Complementary and Alternative Medicine*, *2*(1), 29–32. https://doi.org/10.1093/ecam/neh059

Bankova, V., Castro, S. De, & Marcucci, M. (2000). Propolis: recent advances in chemistry and plant origin. *Apidologie*, *31*(1), 3–15.

Bankova, V., & Marcucci, M. C. (2000). Standardization of propolis: present status and perspectives. *Bee World*, *81*(4), 182–188. https://doi.org/20013063678

Bankova, V., Popova, M., Bogdanov, S., & Sabatini, A. G. (2002). Chemical composition of European propolis: expected and unexpected results. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *57*(5–6), 530–533. https://doi.org/10.1515/znc-2002-5-622

Bankova, V., Popova, M., & Trusheva, B. (2018). The phytochemistry of the honeybee.

Phytochemistry, *155*, 1–11. https://doi.org/10.1016/j.phytochem.2018.07.007

Banskota, A. H., Tezuka, Y., & Kadota, S. (2001). Recent progress in pharmacological research of propolis. *Phytotherapy Research*, *15*(7), 561–571. https://doi.org/10.1002/ptr.1029

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie*, *37*, 1–18. https://doi.org/10.1051/apido

Bononi, M., & Tateo, F. (2008). Liquid chromatography/tandem mass spectrometry analysis of chloramphenicol in propolis extracts available on the Italian market. *Journal of Food Composition and Analysis*, *21*(1), 84–89. https://doi.org/10.1016/j.jfca.2007.08.003

Borrelli, F., Maffia, P., Pinto, L., Ianaro, A., Russo, A., Capasso, F., & Ialenti, A. (2002). Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia*, *73*, S53–S63. https://doi.org/10.1016/S0367-326X(02)00191-0

Bosio, K., Avanzini, C., D'Avolio, A., Ozino, O., & Savoia, D. (2000). In vitro activity of propolis against Streptococcus pyogenes. *Letters in Applied Microbiology*, *31*(2), 174–177. https://doi.org/10.1046/j.1365-2672.2000.00785.x

Bretz, W. A., Paulino, N., Nör, J. E., & Moreira, A. (2014). The effectiveness of propolis on gingivitis: a randomized controlled trial. *The Journal of Alternative and Complementary Medicine*, *20*(12), 943–948. https://doi.org/10.1089/acm.2013.0431

Bromenshenk, J. J., Henderson, C. B., Wick, C. H., Stanford, M. F., Zulich, A. W., Jabbour, R. E., … Cramer, R. A. (2010). Iridovirus and microsporidian linked to honey bee colony decline. *PLoS ONE*, *5*(10), 1–11. https://doi.org/10.1371/journal.pone.0013181

Burdock, G. A. (1998). Review of the biological properties and toxicity of bee propolis (propolis). *Food and Chemical Toxicology*, *36*(4), 347–363. https://doi.org/10.1016/S0278-6915(97)00145-2

Canale, A., Cosci, F., Canovai, R., Giannotti, P., & Benelli, G. (2014). Foreign matter contaminating ethanolic extract of propolis: a filth-test survey comparing products from small beekeeping farms and industrial producers. *Food Additives and Contaminants - Part A*, *31*(12), 2022–2025. https://doi.org/10.1080/19440049.2014.980854

Carvalho, R., Silva-carvalho, R., Baltazar, F., & Almeida-aguiar, C. (2015). Propolis : a complex natural product with a plethora of biological activities that can be explored for drug development. *Evidence-Based Complementary and Alternative Medicine*, *29*, 1–29. https://doi.org/10.1155/2015/206439

Chen, Y. W., Ye, S. R., Ting, C., & Yu, Y. H. (2018). Antibacterial activity of propolins from Taiwanese green propolis. *Journal of Food and Drug Analysis*, *26*(2), 761–768.

https://doi.org/10.1016/j.jfda.2017.10.002

Cheng, P. C., & Wong, G. (1996). Honey bee propolis: prospects in medicine. *Bee World*, *77*, 8–15.

Coelho, V. L. G., Bastos, E. M. A. F., Resende, C. C., Silva, C. M. P. E., Sanches, B. S. F., Castro, F. J. De, … Trindade, O. R. (2007). Brazilian green propolis on Helicobacter pylori infection. A pilot clinical study. *Helicobacter*, *12*(5), 572–574. https://doi.org/10.1111/j.1523-5378.2007.00525.x

Conti, M. E., & Botrè, F. (2001). Honeybees and their products as potential bioindicators of heavy metals contamination. *Environmental Monitoring and Assessment*, *69*(3), 267–282. https://doi.org/10.1023/A:1010719107006

Demestre, M., Messerli, S. M., Celli, N., Shahhossini, M., Kluwe, L., Mautner, V., & Maruta, H. (2009). CAPE (caffeic acid phenethyl ester)-based propolis extract (Bio 30) suppresses the growth of human neurofibromatosis (NF) tumor xenografts in mice. *Phytotherapy Research*, *23*, 226–230.

EC. (2000). *Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin*. *Official Journal of the European Communities* (Vol. L 269). https://doi.org/2004R0726 - v.7 of 05.06.2013

EC. (2011). Commission regulation (EU) No 835/2011 of 19 August 2011 amending regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union*, (215), 4–8. https://doi.org/10.3000/17252555.L_2011.006.eng

EC. (2013). *Evaluation of the CAP Measures Related to Apiculture Agriculture and Rural Development DG- Final Report*.

EFSA. (2013). *Commission Implementing Regulation (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation (EU) No 540/2011*. *Official Journal of the European Union* (Vol. L 139). https://doi.org/10.2903/j.efsa.2013.3067.

Eley, B. M. (1999). Antibacterial agents in the control of supragingival plaque- a review. *British Dental Journal*, *186*(6), 286–296. https://doi.org/10.1038/sj.bdj.4800090a

Ellis, J. D., Klopchin, J., Buss, E., Fishel, F. M., Kern, W. H., Mannion, C., … Webb, S. (2017). *Minimizing honey bee exposure to pesticides*.

Environmental Protection Agency. (2017). Pollinator Protection: Colony Collapse Disorder. Retrieved December 11, 2017, from https://www.epa.gov/pollinator-protection/colony-collapse-disorder

Fearnley, J. (2001). *Bee Propolis: Natural Healing from the Hive*. London: Souvenier Press.

Fernandes-Silva, C. C., Freitas, J. C., Salatino, A., & Salatino, M. L. F. (2013). Cytotoxic Activity of Six Samples of Brazilian Propolis on Sea Urchin (Lytechinus variegatus) Eggs. *Evidence-Based Complementary and Alternative Medicine, 2013*, 1–4. https://doi.org/10.1155/2013/619361

Fernandes, A., Balestrin, E. C., Betoni, J. E. C., De Oliveira Orsi, R., De Souza Da Cunha, M. D. L. R., & Montelli, A. C. (2005). Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. *Memorias Do Instituto Oswaldo Cruz*, *100*(5), 563–566. https://doi.org/10.1590/S0074-02762005000500018

Fernandes, J. A., Leomil, L., Fernandes, A. A. H., & Sforcin, J. M. (2001). The antibacterial activity of propolis produced by Apis mellifera L. and Brazilian stingless bees. *Journal of Venomous Animal Toxins*, *7*, 173–182.

Finger, D., Filho, I. K., Torres, Y. R., & Quina´ıia, S. P. (2014). Propolis as an indicator of environmental contamination by metals. *Bulletin of Environmental Contamination and Toxicology*, *92*, 259–264.

Freitas, S. F., Shinohara, L., Sforcin, J. M., & Guimarães, S. (2006). In vitro effects of propolis on Giardia duodenalis trophozoites. *Phytomedicine*, *13*(3), 170–175. https://doi.org/10.1016/j.phymed.2004.07.008

Friedman, M., & Jürgens, H. S. (2000). Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*(6), 2101–2110. https://doi.org/10.1021/jf990489j

García-Viguera, C., Ferreres, F., & Tomás-B arberán, F. A. (1993). Study of canadian propolis by GC-MS and HPLC. *Zeitschrift Fur Naturforschung*, *48*(9–10), 731–735. https://doi.org/10.1515/znc-1993-9-1009

Gekker, G., Hu, S., Spivak, M., Lokensgard, J. R., & Peterson, P. K. (2005). Anti-HIV-1 activity of propolis in CD4+ lymphocyte and microglial cell cultures. *Journal of Ethnopharmacology*, *102*(2), 158–163. https://doi.org/10.1016/j.jep.2005.05.045

González-Martín, M. I., Revilla, I., Vivar-Quintana, A. M., & Betances Salcedo, E. V. (2017). Pesticide residues in propolis from Spain and Chile: an approach using near infrared spectroscopy. *Talanta*, *165*, 533–539. https://doi.org/10.1016/j.talanta.2016.12.061

Gou, J., Yao, X., Tang, H., Zou, K., Liu, Y., Zuo, H., … Li, Z. (2016). Absorption properties and effects of caffeic acid phenethyl ester and its p-nitro-derivative on P-glycoprotein in Caco-2 cells and rats. *Pharmaceutical Biology*, *54*(12), 2960–2967.

https://doi.org/10.1080/13880209.2016.1197284

Graikou, K., Popova, M., Gortzi, O., Bankova, V., & Chinou, I. (2016). Characterization and biological evaluation of selected Mediterranean propolis samples. is it a new type? *LWT - Food Science and Technology*, *65*, 261–267. https://doi.org/10.1016/j.lwt.2015.08.025

Henshaw, F. R., Bolton, T., Nube, V., Hood, A., Veldhoen, D., Pfrunder, L., … Twigg, S. M. (2014). Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study. *Journal of Diabetes and Its Complications*, *28*(6), 850–857. https://doi.org/10.1016/j.jdiacomp.2014.07.012

https://www.scopus.com/results/results.uri? (2018). Retrieved December 23, 2018, from https://www.scopus.com/results/results.uri?numberOfFields=0&src=s&clickedLink=&edit=&editSaveSearch=&origin=searchbasic&rb_AB=on&authorTab=&affiliationTab=&advancedTab=&scint=1&menu=search&tablin=&searchterm1=propolis&field1=TITLE_ABS_KEY&dateType=Publica

Huang, S., Zhang, C.-P., Wang, K., Li, G. Q., & Hu, F.-L. (2014). Recent advances in the chemical composition of propolis. *Molecules*, *19*(12), 19610–32. https://doi.org/10.3390/molecules191219610

Isla, M. I., Nieva Moreno, M. I., Sampietro, A. R., & Vattuone, M. A. (2001). Antioxidant activity of Argentine propolis extracts. *Journal of Ethnopharmacology*, *76*(2), 165–170. https://doi.org/10.1016/S0378-8741(01)00231-8

Jacques, A., Laurent, M., Ribière-Chabert, M., Saussac, M., Bougeard, S., Budge, G. E., … Chauzat, M. P. (2017). A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. *PLoS ONE*, *12*(3), 1–17. https://doi.org/10.1371/journal.pone.0172591

Kapare, H., Lohidasan, S., Sinnathambi, A., & Mahadik, K. (2017). Standardization, chemical profiling, in vitro cytotoxic effects, in vivo anti-carcinogenic potential and biosafety profile of Indian propolis. *Journal of Ayurveda and Integrative Medicine*, *8*, 1–8. https://doi.org/10.1016/j.jaim.2017.06.003

Kasote, D. M. (2017). Propolis: A neglected product of value in the Indian beekeeping sector. *Bee World*, *94*(3), 80–83. https://doi.org/10.1080/0005772X.2017.1345223

Kasote, D. M., Pawar, M. V., Bhatia, R., Nandre, V. S., Gundu, S. S., Jagtap, S. D., & Kulkarni, M. V. (2017). HPLC, NMR based chemical profiling and biological characterisation of Indian propolis. *Fitoterapia*, *122*, 52–60. https://doi.org/10.1016/j.fitote.2017.08.011

Klaric, I., Pavic, M., Miskulin, I., Blazicevic, V., Dumic, A., & Miskulin, M. (2018). Influence of dietary supplementation of propolis and bee pollen on liver pathology in broiler chickens. *Animals*, *8*(4), 1–10. https://doi.org/10.3390/ani8040054

Koo, H., Rosalen, P. L., Cury, J. a, Park, Y. K., & Bowen, W. H. (2002). Effects of Compounds Found in Propolis on Streptococcus mutans growth and on glucosyltransferase activity. *Antimicrobial Agents and Chemotherapy*, *46*(5), 1302–1309. https://doi.org/10.1128/AAC.46.5.1302

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., & Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology*, *64*(3), 235–240. https://doi.org/10.1016/S0378-8741(98)00131-7

Kumar, A., Musalaiah, S. V. V. S., Pantareddy, I., & Sudhakar, S. (2015). Comparison of plaque inhibiting efficacies of aloe vera and propolis tooth gels: A randomized PCR study. *Journal of Clinical and Diagnostic Research*, *9*(9), ZC01-ZC03. https://doi.org/10.7860/JCDR/2015/13185.6413

Kuropatnicki, A. K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–11. https://doi.org/10.1155/2013/964149

Levy, S. B. (1992). How Miracle Drugs Are Destroying the Miracle. In *The Antibiotic Paradox* (1st ed., p. XIV, 279). Springer US. https://doi.org/10.1007/978-1-4899-6042-9

Liu, J., Liu, Y., Wang, Y., Abozeid, A., Zu, Y. G., & Tang, Z. H. (2017). The integration of GC–MS and LC–MS to assay the metabolomics profiling in Panax ginseng and Panax quinquefolius reveals a tissue- and species-specific connectivity of primary metabolites and ginsenosides accumulation. *Journal of Pharmaceutical and Biomedical Analysis*, *135*, 176–185. https://doi.org/10.1016/j.jpba.2016.12.026

Maraschin, M., Somensi-Zeggio, A., Oliveira, S. K., Kuhnen, S., Tomazzoli, M. M., Raguzzoni, J. C., … Rocha, M. (2016). Metabolic Profiling and Classification of Propolis Samples from Southern Brazil: An NMR-Based Platform Coupled with Machine Learning. *Journal of Natural Products*, *79*(1), 13–23. https://doi.org/10.1021/acs.jnatprod.5b00315

Marcucci, M. C. (1994). Propolis : chemical composition , biological properties and therapeutic activity. *Apidologie*, *26*(1), 83–99. https://doi.org/10.1051/apido:19950202

Markham, K. R., Mitchell, K. a., Wilkins, A. L., Daldy, J. a., & Lu, Y. (1996). HPLC and GC-MS identification of the major organic constituents in New Zeland propolis.

Phytochemistry, *42*(1), 205–211. https://doi.org/10.1016/0031-9422(96)83286-9

Massaro, C. F., Simpson, J. B., Powell, D., & Brooks, P. (2015). Chemical composition and antimicrobial activity of honeybee (Apis mellifera ligustica) propolis from subtropical eastern Australia. *Science of Nature*, *102*, 68. https://doi.org/10.1007/s00114-015-1318-z

Matin, G., Kargar, N., & Buyukisik, H. B. (2016). Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. *Ecological Engineering*, *90*, 331–335. https://doi.org/10.1016/j.ecoleng.2016.01.035

Monti, M., Berti, E., Carminati, G., & Cusini, M. (1983). Occupational and cosmetic dermatitis from propolis. *Contact Dermatitis*, *9*(2), 163.

Moret, S., Purcaro, G., & Conte, L. S. (2010). Polycyclic aromatic hydrocarbons (PAHs) levels in propolis and propolis-based dietary supplements from the Italian market. *Food Chemistry*, *122*(1), 333–338. https://doi.org/10.1016/j.foodchem.2010.02.041

Mujica, V., Orrego, R., Pérez, J., Romero, P., Ovalle, P., Zúñiga-Hernández, J., … Leiva, E. (2017). The role of propolis in oxidative stress and lipid metabolism: A randomized controlled trial. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–11. https://doi.org/10.1155/2017/4272940

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., VanEngelsdorp, D., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in north American apiaries: implications for honey bee health. *PLoS ONE*, *5*(3), e9754. https://doi.org/10.1371/journal.pone.0009754

Murad, J. M., Calvi, S. A., Soares, A. M. V. C., Bankova, V., & Sforcin, J. M. (2002). Effects of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against Paracoccidioides brasiliensis. *Journal of Ethnopharmacology*, *79*(3), 331–334. https://doi.org/10.1016/S0378-8741(01)00404-4

Narita, Y., & Inouye, K. (2013). Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. *Journal of Agricultural and Food Chemistry*, *61*(4), 966–972. https://doi.org/10.1021/jf304105w

Ni, Y., Su, M., Qiu, Y., Chen, M., Liu, Y., Zhao, A., & Jia, W. (2007). Metabolic profiling using combined GC-MS and LC-MS provides a systems understanding of aristolochic acid-induced nephrotoxicity in rat. *FEBS Letters*, *581*(4), 707–711. https://doi.org/10.1016/j.febslet.2007.01.036

Niell, S., Jesús, F., Pérez, C., Mendoza, Y., Díaz, R., Franco, J., … Heinzen, H. (2015). QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. *Journal of Agricultural and Food Chemistry*, *63*(18), 4484–4492. https://doi.org/10.1021/acs.jafc.5b00795

Oellig, C. (2016). Acetonitrile extraction and dual-layer solid phase extraction clean-up for pesticide residue analysis in propolis. *Journal of Chromatography A*, *1445*, 19–26. https://doi.org/10.1016/j.chroma.2016.03.082

Oršolić, N., Knežević, A. H., Šver, L., Terzić, S., & Bašić, I. (2004). Immunomodulatory and antimetastatic action of propolis and related polyphenolic compounds. *Journal of Ethnopharmacology*, *94*(2–3), 307–315. https://doi.org/10.1016/j.jep.2004.06.006

Oruç, H. H., Sorucu, A., Ünal, H. H., & Aydin, L. (2017). Effects of season and altitude on biological active certain phenolic compounds levels and partial standardization of propolis. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *64*, 13–20.

Ota, C., Unterkircher, C., Fantinato, V., & Shimizu, M. T. (2001). Antifungal activity of propolis on different species of Candida arten. *Mycoses*, *44*(9–10), 375–8.

Papotti, G., Bertelli, D., Bortolotti, L., & Plessi, M. (2012). Chemical and functional characterization of Italian propolis obtained by different harvesting methods. *Journal of Agricultural and Food Chemistry*, *60*(11), 2852–2862. https://doi.org/10.1021/jf205179d

Park, Y. K., Alencar, S. M., & Aguiar, C. L. (2002). Botanical origin and chemical composition of Brazilian propolis. *Journal of Agriculture and Food Chemistry*, *50*, 2502–2056. https://doi.org/10.1021/jf011432b

Pina, G. D. M. S., Lia, E. N., Berretta, A. A., Nascimento, A. P., Torres, E. C., Buszinski, A. F. M., … Martins, V. D. P. (2017). Efficacy of propolis on the denture stomatitis treatment in older adults: A multicentric randomized trial. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–9. https://doi.org/10.1155/2017/8971746

Piredda, M., Facchinetti, G., Biagioli, V., Giannarelli, D., Armento, G., Tonini, G., & De Marinis, M. G. (2017). Propolis in the prevention of oral mucositis in breast cancer patients receiving adjuvant chemotherapy: A pilot randomised controlled trial. *European Journal of Cancer Care*, *26*(6), 1–8. https://doi.org/10.1111/ecc.12757

Pollinator Protection. (2017). Retrieved November 28, 2017, from https://www.epa.gov/pollinator-protection/colony-collapse-disorder

Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A. G., … Bogdanov, S. (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. *Phytochemical Analysis*, *15*(4), 235–340. https://doi.org/10.1002/pca.777

Popova, M., Giannopoulou, E., Skalicka-Woźniak, K., Graikou, K., Widelski, J., Bankova, V., … Chinou, I. (2017). Characterization and biological evaluation of propolis from Poland. *Molecules*, *22*(7), 1159. https://doi.org/10.3390/molecules22071159

Popova, M. P., Graikou, K., Chinou, I., & Bankova, V. S. (2010). GC-MS profiling of diterpene compounds in mediterranean propolis from Greece. *Journal of Agricultural and Food Chemistry*, *58*(5), 3167–3176. https://doi.org/10.1021/jf903841k

Popova, M. P., S.Bankova, V., Bogdanov, S., Tsvetkovac, I., Naydenskic, C., Marcazzand, G. L., & Sabatini, A.-G. (2007). Chemical characteristics of poplar type propolis of different geographic origin. *Apidologie*, *38*, 306–311. https://doi.org/10.1051/apido

Prabhakar, A. R., Karuna, Y. M., & Deepak, B. M. (2015). Cavity disinfection in minimally invasive dentistry - comparative evaluation of Aloe vera and propolis: A randamized clinical trial. *Contemporary Clinical Dentistry*, *6*(5), 24–31.

Raghukumar, R., Vali, L., Watson, D., Fearnley, J., & Seidel, V. (2010). Antimethicillin-resistant Staphylococcus aureus (MRSA) activity of ‘ Pacific propolis ’ and isolated prenylflavanones. *Phytotherapy Research*, *24*(8), 1181–1187. https://doi.org/10.1002/ptr.3096

Sá-Nunes, A., Faccioli, L. H., & Sforcin, J. M. (2003). Propolis: lymphocyte proliferation and IFN-γ production. *Journal of Ethnopharmacology*, *87*(1), 93–97. https://doi.org/10.1016/S0378-8741(03)00121-1

Sahinler, N., & Kaftanoglu, O. (2005). Natural product propolis: chemical composition. *Natural Product Research*, *19*(2), 183–188. https://doi.org/10.1080/14786410410001704877

Salatino, A., Fernandes-Silva, C. C., Righi, A. A., & Salatino, M. L. F. (2011). Propolis research and the chemistry of plant products. *Natural Product Reports*, *28*(5), 925–936. https://doi.org/10.1039/c0np00072h

Sales, A., Alvarez, A., Areal, M. R., Maldonado, L., Marchisio, P., Rodríguez, M., & Bedascarrasbure, E. (2006). The effect of different propolis harvest methods on its lead contents determined by ET AAS and UV-visS. *Journal of Hazardous Materials*, *137*(3), 1352–1356. https://doi.org/10.1016/j.jhazmat.2006.05.026

Samet, N., Laurent, C., Susarla, S. M., & Samet-Rubinsteen, N. (2007). The effect of bee

propolis on recurrent aphthous stomatitis: A pilot study. *Clinical Oral Investigations*, *11*(2), 143–147. https://doi.org/10.1007/s00784-006-0090-z

Sanpa, S., Popova, M., Bankova, V., Tunkasiri, T., Eitssayeam, S., & Chantawannakul, P. (2015). Antibacterial compounds from propolis of Tetragonula laeviceps and Tetrigona melanoleuca (Hymenoptera: Apidae) from Thailand. *PLoS ONE*, *10*(5), 1–11. https://doi.org/10.1371/journal.pone.0126886

Santos, F. A., Bastos, E. M. A., Uzeda, M., Carvalho, M. A. R., Farias, L. M., Moreira, E. S. A., & Braga, F. C. (2002). Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. *Journal of Ethnopharmacology*, *80*(1), 1–7. https://doi.org/10.1016/S0378-8741(02)00003-X

Sawaya, A. C. H. F., Calado, J. C. P., Santos, L. C. dos, Marcucci, M. C., Akatsu, I. P., Soares, A. E. E., … Eberlin, M. N. (2009). Composition and antioxidant activity of propolis from three species of Scaptotrigona stingless bees. *Journal of ApiProduct and ApiMedical Science*, *1*(2), 37–42. https://doi.org/10.3896/ibra.4.01.2.03

Sawaya, A. C. H. F., Cunha, I. B. S., Marcucci, M. C., Aidar, D. S., Silva, E. C. A., Carvalho, C. A. L., & Eberlin, M. N. (2007). Electrospray ionization mass spectrometry fingerprinting of propolis of native Brazilian stingless bees. *Apidologie*, *38*, 93–103. https://doi.org/10.1051/apido:2006058

Sawaya, A. C. H. F., Tomazela, D. M., Cunha, I. B. S., Bankova, V. S., Marcucci, M. C., Custodio, A. R., & Eberlin, M. N. (2004). Electrospray ionization mass spectrometry fingerprinting of beer. *The Analyst*, *129*(6), 739–744. https://doi.org/10.1039/b415252b

Scazzocchio, F., D'Auria, F. D., Alessandrini, D., & Pantanella, F. (2006). Multifactorial aspects of antimicrobial activity of propolis. *Microbiological Research*, *161*(4), 327–333. https://doi.org/10.1016/j.micres.2005.12.003

Seidel, V., Peyfoon, E., Watson, D. G., & Fearnley, J. (2008). Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. *Phytotherapy Research: PTR*, *22*(9), 1256–1263. https://doi.org/10.1002/ptr.2480

Seo, K. W., Park, M., Song, Y. J., Kim, S. J., & Yoon, K. R. (2003). The protective effects of propolis on hepatic injury and its mechanism. *Phytotherapy Research*, *17*(3), 250–253. https://doi.org/10.1002/ptr.1120

Sforcin, J. M. (2007). Propolis and the immune system: a review. *Journal of Ethnopharmacology*, *113*(1), 1–14. https://doi.org/10.1016/j.jep.2007.05.012

Sforcin, J. M., & Bankova, V. (2011). Propolis: Is there a potential for the development of new drugs? *Journal of Ethnopharmacology*, *133*(2), 253–260.

https://doi.org/10.1016/j.jep.2010.10.032

Sforcin, J. M., Fernandes, A., Lopes, C. A. M., Bankova, V., & Funari, S. R. C. (2000). Seasonal effect on Brazilian propolis antibacterial activity. *Journal of Ethnopharmacology*, *73*(1–2), 243–249. https://doi.org/10.1016/S0378-8741(00)00320-2

Silici, S., & Kutluca, S. (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *Journal of Ethnopharmacology*, *99*(1), 69–73. https://doi.org/10.1016/j.jep.2005.01.046

Silva Cunha, I. B. da, Salomão, K., Shimizu, M., Bankova, V. S., Custódio, A. R., Castro, S. L. de, & Marcucci, M. C. (2004). Antitrypanosomal activity of Brazilian propolis from Apis mellifera. *Chemical & Pharmaceutical Bulletin*, *52*(5), 602–604. https://doi.org/10.1248/cpb.52.602

Soroy, L., Bagus, S., & Yongkie, I. P. (2014). The effect of a unique propolis compound (propoelixTM) on clinical outcomes in patients with dengue hemorrhagic fever. *Infection and Drug Resistance*, *7*, 323–329. https://doi.org/10.2147/IDR.S71505

Stepanović, S., Antić, N., Dakić, I., & Svabić-Vlahović, M. (2003). In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microbiological Research*, *158*, 353–357. https://doi.org/10.1078/0944-5013-00215

Tang, T. X., Guo, W. Y., Xu, Y., Zhang, S. M., Xu, X. J., Wang, D. M., … Yang, D. P. (2014). Thin-layer chromatographic identification of chinese propolis using chemometric fingerprinting. *Phytochemical Analysis*, *25*(3), 266–272. https://doi.org/10.1002/pca.2502

Tomaževič, T., & Jazbec, J. (2013). A double blind randomised placebo controlled study of propolis (bee glue) effectiveness in the treatment of severe oral mucositis in chemotherapy treated children. *Complementary Therapies in Medicine*, *21*(4), 306–312. https://doi.org/10.1016/j.ctim.2013.04.002

Toreti, V. C., Sato, H. H., Pastore, G. M., & Park, Y. K. (2013). Recent progress of propolis for its biological and chemical compositions and its botanical origin. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *2013*, 1–13. https://doi.org/10.1155/2013/697390

Torwane, N. A., Hongal, S., Goel, P., Chandrashekhar, B. R., Jain, M., & Saxena, E. (2013). A clinical efficacy of 30% ethanolic extract of Indian propolis and Recaldent in management of dentinal hypersensitivity: A comparative randamized clinical trail. *Europian Journal of Dentstry*, *7*(4), 461–468.

Tulsani, S. G., Chikkanarasaiah, N., Siddaiah, S. B., & Krishnamurthy, N. H. (2014). The effect of propolis and xylitol chewing gums on salivary Streptococcus mutant count: A clinical trail. *Indian Journal of Dental Research*, *25*(6), 737–741.

Vujić, M., & Pollak, L. (2015). Composition, labelling, and safety of food supplements based on bee products in the legislative framework of the European Union - Croatian experiences. *Arhiv Za Higijenu Rada i Toksikologiju*, *66*(4), 243–249. https://doi.org/10.1515/aiht-2015-66-2654

Watson, D. G., Peyfoon, E., Zheng, L., Lu, D., Seidel, V., Jonhston, B., … Fearnley, J. (2006). Application of principal components analysis to 1H-NMR data obtained from propolis samples of different geographical origin. *Phytochemical Analysis*, *17*(5), 323–331. https://doi.org/10.1002/pca.921

Wojtyczka, R. D., Dziedzic, A., Idzik, D., Kepa, M., Kubina, R., Kabała-Dzik, A., … Wasik, T. J. (2013). Susceptibility of Staphylococcus aureus clinical isolates to propolis extract alone or in combination with antimicrobial drugs. *Molecules*, *18*(8), 9623–9640. https://doi.org/10.3390/molecules18089623

Yildirim, Z., Hacievliyagil, S., Kutlu, N. O., Aydin, N. E., Kurkcuoglu, M., Iraz, M., & Durmaz, R. (2004). Effect of water extract of Turkish propolis on tuberculosis infection in guinea-pigs. *Pharmacological Research*, *49*(3), 287–292. https://doi.org/10.1016/j.phrs.2003.10.007

Yuliana, N. D., Wijaya, C. H., & Nasrullah, N. (2013). Classification of Trigona Spp Bee Propolis from Four Regions in Indonesia using FTIR based Metabolomic Approach. In *13th ASEAN Food Conference, 9-11 September 2013, Singapore* (pp. 9–11).

Zelinkova, Z., & Wenzl, T. (2015). EU marker polycyclic aromatic hydrocarbons in food supplements: analytical approach and occurrence. *Food Additives and Contaminants - Part A*, *32*(11), 1914–1926. https://doi.org/10.1080/19440049.2015.1087059

Zhang, C. P., Ping, S., Wang, K., Huang, S., & Hu, F. L. (2015). A survey of the incidence of poplar tree gum in propolis products on the Chinese retail market. *Journal of Apicultural Research*, *54*(1), 30–35. https://doi.org/10.1080/00218839.2015.1029784

Zhang, L. H., Li, Y., Yuan, L., & Zhang, C. J. (2011). Adsorption and removal of lead in propolis with modified rushes. *ISWREP 2011 - Proceedings of 2011 International Symposium on Water Resource and Environmental Protection*, *3*, 2021–2024. https://doi.org/10.1109/ISWREP.2011.5893657

Zhou, J., Xue, X., Li, Y., Zhang, J., Chen, F., Wu, L., … Zhao, J. (2009). Multiresidue determination of tetracycline antibiotics in propolis by using HPLC-UV detection with

ultrasonic-assisted extraction and two-step solid phase extraction. *Food Chemistry*, *115*(3), 1074–1080. https://doi.org/10.1016/j.foodchem.2008.12.031

Zhu, W., Schmehl, D. R., Mullin, C. A., & Frazier, J. L. (2014). Four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honey bee larvae. *PLoS ONE*, *9*(1), e77547. https://doi.org/10.1371/journal.pone.0077547